

the evanescent field, and can be interpreted as a density or a refractive index of the cellular material. The quantitative nature of SPR images and the direct relationship to refractive index changes at the surface sensor allow for visualization new insights into mechanisms of cell biology at an interface. When applied to mammalian cells, such as rat aortic smooth muscle cells, cellular components near the sensor surface such as the cell membrane, focal adhesions, and cell nucleus are visualized in the SPRI image. Focal adhesion sizes measured by SPRI are similar with those highlighted with fluorescent antibody stained vinculin. In addition, a positive correlation between focal adhesion size and protein density is observed by SPR imaging. When SPRI is applied to pathogenic biofilms of *Streptococcus mutans*, distinct components of the bacterial biofilm at the surface including individual bacteria, bacterial microcolonies, and extracellular polymeric substance (EPS) are observed. SPRI shows that the refractive index of bacteria in a biofilm increases over time compared to that of bacteria not in a biofilm, which remains constant. SPRI also indicates that the EPS material generated in the biofilm at early time points is thicker near the bacterial microcolony periphery. This suggests that the EPS matrix is generated at the colony edge and that SPRI can be used to monitor the dynamics of EPS production in biofilms.

#### 1635-Pos Board B586

##### Light Sheet Fluorescence Microscopy (LSFM) for Two-Photon Excitation Imaging of Thick Samples

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Over the last decades, fluorescence microscopy techniques have been developed in order to provide a deeper, faster and higher resolution imaging of three-dimensional biological samples. Within this framework, Light Sheet Fluorescence Microscopy (LSFM) became an increasingly useful and popular imaging technique able to answer several biological questions in the field of developmental biology [1]. Thanks to the spatial confinement of the excitation process within a thin sheet in the focal plane, it provides an intrinsic optical sectioning and a reduced phototoxicity. On the other side, Two-Photon Excitation (2PE), thanks to the use of IR wavelengths, has become an invaluable tool to improve imaging capabilities in terms of imaging depth and spatial resolution [2,3].

In this work we tested and compared the advantages provided by Two Photon Excitation in combination with two different light sheet based architectures: Selective Plane Illumination Microscopy (SPIM) and Inverted Selective Plane Illumination Microscopy (iSPIM) [4]. The two different optical approaches are characterized in terms of illumination intensity distributions and in terms of point spread function measurements, both in the linear and non linear regime. Additionally, particular attention has been addressed to the relationship between the sample holder and the specific sample geometry, showing the suitability of the inverted configuration when the sample geometry does not allow embedding in agarose gel (for example brain slices and retina). Furthermore, TPE-SPIM has been tested towards live imaging of nervous system in small animals, such as *Danio Rerio*.

[1] Huisken J. et al. Science. 305(5686):1007-9, 2004.

[2] Truong T.V. et al Nat Methods 8(9):757-760, 2011.

[3] Lavagnino Z. et al. Optics Express 21(5):5998-6008, 2013.

[4] Wu Y. et al. PNAS 108(43):17708-13, 2011.

## Biosensors I

#### 1636-Pos Board B587

##### Sensing Elements Encapsulated within Hydrogel Matrix to Enhance the Signal-To-Noise Ratio

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In this study, we developed polydiacetylene(PDA) vesicle based colorimetric biosensor. PDA vesicle is a cluster of diacetylene(DA) lipid that has a unique property of color transition from blue-to-red upon external stimuli, such as temperature, pH, and mechanical stress. In our biosensor, PDA vesicle was conjugated with target-specific antibody by EDC/NHS coupled reaction to identify target protein. Antibody conjugated PDA vesicle was then encapsulated into Poly(ethylene glycol) diacrylate(PEG-DA) hydrogel at high density to enhance reaction sensitivity. Furthermore, PDA vesicle encapsulating hydrogel was formed in spherical shape by using droplet-microfluidic device to enable trans-

portation. We named this colorimetric biosensor as immunohydrogel bead. Detection of PAT protein from genetically modified organisms (GMO) was attempted for practical use. The PAT protein allows herbicide resistance to GMO and was known as model protein of GMO. Using immunohydrogel bead, PAT protein was detected to 20 nM with naked eyes which means that even 1% of GMO can be detected. Thus, we expect that immunohydrogel bead can be used practically not only in GMO but also in other targets.

#### 1637-Pos Board B588

##### Simulation Results for an Optically Active Semiconductor Nanopore

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Optical molecule detection is of increasing interest in biophysical applications especially for high speed molecule detection and DNA sequencing. We present an optically active solid-state nanopore design for probing charged molecules. The proposed semiconductor nanopore (SNP) is a hollow cylindrical semiconductor heterostructure consisting of a low band gap semiconductor in the center surrounded by a high band gap semiconductor. We use a single sub-band approximation to simulate the behavior of these SNPs and show that this structure exhibit a high quantum confinement effect that can be tuned by geometrical modification.

Our simulations indicate that a charge distribution within the SNP can change the energy state of the pore significantly. This effect is proposed to be usable for particle detection.

#### 1638-Pos Board B589

##### Lipid Bilayer Coated Nanopipettes as Generic Nanopore Sensors with Enhanced Functionality

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Nanopore sensors show great promise for use as single-molecule diagnostic devices. Current limitations to nanopores include laborious fabrication, undesired interactions between species in solution and the nanopore walls, pore clogging and difficulties in controlling the speed of translocations.

Work in the literature shows the viability of using lipid coated solid-state pores and nanopipettes to overcome some of the aforementioned limitations to the development of nanopore biosensors.<sup>1,2</sup> Such coatings offer increased control over the surface charge of the pore and the translocation speed, preconcentration of analytes on the pore surface prior to analysis, enhanced specificity and a reduction in pore clogging.

Our work revolves around developing lipid-bilayer modified pores as a generic platform to detect epigenetic modifications of DNA. Compared to pull-down assays, the combination with nanopore analysis provides enhanced information content, while being faster and cheaper than single-base resolution bisulfite sequencing. Our data further show that the coated nanopipettes remain stable for hours and suitable for DNA translocation experiments. DNA methylation is being investigated as a model system, with particular relevance as a biomarker for early cancer diagnosis.

1. Yusko, E. C. et al. Controlling protein translocation through nanopores with bio-inspired fluid walls. Nat. Nanotechnol. 6, 253-260 (2011).

2. Hernandez-Ainsa, S. et al. Lipid-coated nanocapillaries for DNA sensing. Analyst 138, 104-106 (2013).

#### 1639-Pos Board B590

##### Polarization-Based DNA Sandwich Assay with Au Nanoparticles using the Influence of Inter-Particle Distance

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Nucleic acid hybridization assay is widely used for application in biomedical research. In this field there is a considerable demand for the development of more sensitive, quantitative, rapid and low-cost method for target detection. To meet these requirements, Nanoparticle-based controlled assembly method via DNA has become a useful tool for clinical diagnostics because of their strict selectivity. Gold nanoparticles(AuNPs) can be synthesized amenably and can be made highly stable. Moreover, they have unique optical response of surface plasmon resonance and this property can be easily adjusted by varying their size, shape, and the surrounding chemical environment. In this research, we focus inter-particle distance of AuNP dimer. Inter-particle separations are greatly sensitive for the resonance wavelength and interaction between AuNP. When it is small(<2nm), it can be observed that specific optical response in occurred such as significantly high intensity scattering, peak shift of absorbance spectra.

Recently, plasma mass spectrometry and surface enhanced raman spectroscopy(SERS) were reported as more sensitive method. However, they need